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Somatocrinin in cattle

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Somatocrinin in cattle

H. LAPIERRE, D. PETITCLERC, and G. PELLETIER
Research Station
Lennoxville, Quebec

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SUMMARY

Somatocrinin (or growth hormone-releasing factor, GRF) is the hypothalamic factor which stimulates the secretion of somatotropin (ST, or growth hormone, GH) by the pituitary gland. Since the early 1980s, when it became possible to produce ST through the use of recombinant DNA technology, a number of studies have demonstrated its value in improving the animal performance of cattle. GRF was first sequenced in the early 1980s. Shortly thereafter, the Lennoxville Research Station developed a method involving the administration of exogenous GRF to increase endogenous secretion of ST as a mean of improving animal performance. This document describes the progress achieved to date by researchers at Lennoxville working on GRF in cattle since its characterization in 1982. First, a number of experiments were conducted to determine the optimal route and dose of GRF required to produce significant increases in ST concentrations. The next step was to determine whether the increase in ST concentrations following the administration of GRF was sufficient to increase milk production. The initial 10-day studies on milk production showed increased yields of 15%, or 2 to 3 kg of milk per day. These initial studies were conducted using the original GRF molecule, which contains 44 amino acids. Research on rats had shown that the fragment containing the first 29 amino acids had the same biological activity as the parent molecule. The GRF(1-29)NH_2 fragment was therefore selected for study in cattle. In cows, the (1-29)NH_2 fragment was as potent as (1-44)NH_2 on ST secretion and milk production. Therefore, in an effort to reduce the doses required and to increase ST concentrations still further, an analogue containing three amino acid substitutions was used: it proved to be 16 times more potent than GRF(1-29)NH_2 in stimulating ST secretion and milk production. Finally, if it is to be of commercial value, GRF must induce ST secretion over extended treatment periods. Studies involving the administration of GRF for 60 or 182 days showed that it maintained its effect on ST secretion and increased milk production.

In growing cattle, GRF increased dietary digestibility and nitrogen retention. It did not, however, affect the animal's total energy retention; instead, it diverted this energy towards protein and away from fat. In heifers, GRF increased the volume of parenchymal epithelial tissue in the mammary gland.

In little more than 10 years, GRF has advanced from the stage of biochemical characterization to a demonstrated potential for use in improving animal performance of cattle. Further research will be required to determine the exact mechanisms by which it works and the various signals involved in the metabolic coordination permitting these increases in production efficiency.

RÉSUMÉ

La somatocrinine (GRF) est le facteur hypothalamique qui stimule la sécrétion de la somatotrophine (ST ou hormone de croissance) par l'hypophyse. Depuis le début des années 80 où il est devenu possible de fabriquer la ST par ADN recombinant, plusieurs expériences ont démontré que son utilisation permet d'augmenter les performances zootechniques des bovins. Au début des années 80, la séquence du GRF a été caractérisée. À la Station de recherches de Lennoxville, nous avons alors développé l'approche utilisant l'apport exogène de GRF afin d'augmenter les sécrétions endogènes de ST comme moyen d'améliorer les performances des animaux. Le présent bulletin décrit le cheminement que le groupe de scientifiques de Lennoxville oeuvrant sur le GRF chez le bovin a suivi, depuis que le GRF a été caractérisé en 1982 jusqu'à maintenant. Quelques expériences ont tout d'abord été réalisées afin de déterminer la voie et la dose de GRF à administrer afin d'augmenter de façon significative les concentrations de ST. Il a ensuite fallu déterminer si l'augmentation de ST suite à l'administration de GRF était suffisante pour augmenter la production laitière. Les premières expériences sur la production laitière, d'une durée de 10 jours, ont entraîné des augmentations de production de l'ordre de 15 %, soit de 2 à 3 kg de lait par jour. Ces premières expériences ont été effectuées avec la molécule originale de GRF qui contient 44 acides aminés. Des travaux chez le rat avaient démontré que le fragment contenant les 29 premiers acides aminés était biologiquement aussi actif que la molécule mère. Le fragment GRF(1-29)NH_2 a ainsi été étudié chez le bovin. Chez la vache, le fragment (1-29)NH_2 a été aussi puissant que le (1-44)NH_2 sur la sécrétion de ST et sur la production laitière. Aussi, afin de diminuer les doses utilisées et d'augmenter encore davantage les concentrations de ST, un analogue comportant trois substitutions d'acides aminés a été utilisé: il a été 16 fois plus puissant que le GRF(1-29)NH_2 pour stimuler la sécrétion de ST et la production laitière. Finalement, pour avoir un intérêt pratique, le GRF doit pouvoir stimuler la sécrétion de ST pendant de longues périodes de traitement. Le GRF administré pendant 60 ou 182 jours a maintenu son effet sur la sécrétion de ST et a augmenté la production laitière.

Chez le bovin en croissance, le GRF a augmenté la digestibilité de la ration et la rétention azotée. Il n'a cependant pas influencé l'énergie totale retenue par l'animal mais a modifié la répartition de cette énergie vers les protéines au détriment des lipides. Chez la génisse, le GRF a augmenté le volume du tissu épithélial parenchymateux de la glande mammaire.

Ainsi, depuis à peine dix ans, le GRF est passé de la caractérisation biochimique à la démonstration de son potentiel d'utilisation pour améliorer les performances zootechniques des bovins. Il reste encore à préciser ses mécanismes d'action et les différents signaux qui participent à la coordination métabolique permettant ces augmentations de l'efficacité de la production.

1. INTRODUCTION

USE OF SOMATOCRININ - PHYSIOLOGICAL BASIS

The regulation of body functions is controlled by the nervous and endocrine systems. In general, the nervous system serves to regulate those functions requiring rapid adjustment, while the endocrine system controls long-term processes, such as growth, reproduction and lactation.

1.1 THE ENDOCRINE SYSTEM

The endocrine system consists of a variety of glands located in different parts of the body and specializing in the production of hormones. By definition, hormones are chemical agents synthesized in specific parts of the body, usually specialized glands producing internal secretions. Following synthesis, the hormones are transported by the blood to the specific target organs or tissues on which they act. Specific receptors on each target cell ensure that each hormone acts in a very specific manner. The receptors of the protein hormones, such as prolactin and somatotropin, are located on the external surface of the cell membrane. Once formed, the hormone-receptor complex becomes a single entity responsible at each binding site for the biological actions characteristic of the hormone, such as cell multiplication, organelle formation, synthesis of DNA, RNA or proteins and protein phosphorylation.

A certain functional coordination exists between the nervous system and the endocrine system. This function is performed by the neuroendocrine system. The central organ of the neuroendocrine system is the part of the brain known as the hypothalamus, which acts directly on the anterior and posterior pituitary. The neurons of the hypothalamus at the base of the brain synthesize stimulatory or inhibitory hormone factors. Some of these factors are then released into the hypothalamo-pituitary portal circulation which supplies the anterior pituitary, thus regulating its hormone secretion to the peripheral circulation. For example, secretions of somatotropin (or growth hormone) are controlled primarily by two hypothalamic factors: somatostatin, which inhibits the release of somatotropin by the somatotrophic cells of the pituitary gland, and somatocrinin, which stimulates it. We shall return to this subject later in the text.

1.2 PHYSIOLOGY OF LACTATION

A cow's milk production potential is dependent on the development of its mammary gland (mammogenesis), the milk-synthesizing capacity of the secretory cells (lactogenesis) and the cow's ability to sustain an existing milk flow (galactopoiesis). Numerous hormones are involved in the control of mammary development in ruminants. The most significant are prolactin and somatotropin secreted by the pituitary gland, estrogens and progesterone secreted by the ovaries and the lactogenic hormone produced by the placenta. Estrogens and somatotropin stimulate the growth of the lacteal ducts, while progesterone and

prolactin promote the growth of lobulo-alveolar tissue. Without the pituitary hormones, steroids are completely ineffective. The corticosteroids secreted by the adrenal gland increase the potential effects of these mammogenic hormones. However, the mammary development produced by a combination of all these hormones is comparable only to that of mid-gestation. It is the lactogenic placental hormone which is believed to stimulate the maximal development of the mammary gland in the second half of gestation. This action on the part of the lactogenic placental hormone is believed to be due to biological properties similar to those of prolactin and somatotropin.

Lactogenesis is the process of cell differentiation by which the alveolar cells of the mammary gland acquire the ability to synthesize milk. The term lactogenic describes the factors responsible for the initiation of milk secretion in late gestation and at parturition. The minimum hormonal requirements for lactogenesis are increased secretions of prolactin, corticotropin, which stimulate secretion of the glucocorticoids, and estrogens, and a relative absence of progesterone.

The secretion of milk, or lactation, involves the intracellular synthesis of the components of milk in the alveolar epithelial cells and their subsequent passage from the cellular cytoplasm to the alveolar luminal space. The milk is then discharged from the mammary gland during lactation or milking. Continued lactation, or galactopoiesis, requires maintenance of the number and synthetic activity of the alveolar cells, together with the efficiency of the milk ejection reflex. The hormones required to maintain the number and activity of the alveolar cells are prolactin, somatotropin, the glucocorticoids, the thyroid hormones, insulin and the parathyroid hormones. Oxytocin, in turn, is essential for milk ejection.

1.3 HORMONES

The word hormone has an unfortunate connotation among consumers. It is associated with hormone-treated chicken, doping in amateur sport, sex, etc. Hormone-treated chicken is merely an expression, since chickens today are not given hormones to stimulate their growth, folk wisdom to the contrary. Hormones are not alone in having a poor reputation among consumers. They are followed, in decreasing order, by antibiotics, residues and pathogenic organisms as the factors most feared by consumers in their food. When researchers are asked the same question, the order of priority is reversed: pathogens are at the top of the list, with hormones a distant last.

In fact, in all mammals, including man and all domestic species, hormones are merely organic chemical substances synthesized by specialized cells within the body, whose role is to coordinate and regulate cell activity. Without hormones, there would be no body growth, no milk production ... no life.

2. SOMATOTROPIN

Somatotropin (ST), also known as growth hormone, is a natural protein present endogenously in all animal species, including man. The secretion of ST by the pituitary into the bloodstream is regulated by the brain (hypothalamus), which releases two control factors: somatocrinin or growth hormone-releasing factor (GRF), which stimulates the secretion of ST, and somatostatin, which inhibits it (figure 1). GRF acts specifically on ST, while somatostatin influences the secretion of a number of hormones. Somatostatin, a powerful inhibitor of ST secretion, was first discovered by Krulich and collaborators (1968) and subsequently characterized by Brazeau and collaborators (1973). Guillemin, Brazeau et al. (1982) used tumours of the human pancreas to isolate and sequence three molecules showing GRF activity: GRF(1-37)OH, GRF(1-40)OH and GRF(1-44)NH_2. In 1984, Guillemin and collaborators indicated that the GRF from human hypothalamus cells was identical to the GRF(1-44)NH_2 molecule isolated from tumours of pancreatic cells of human origin (Guillemin et al. 1984).

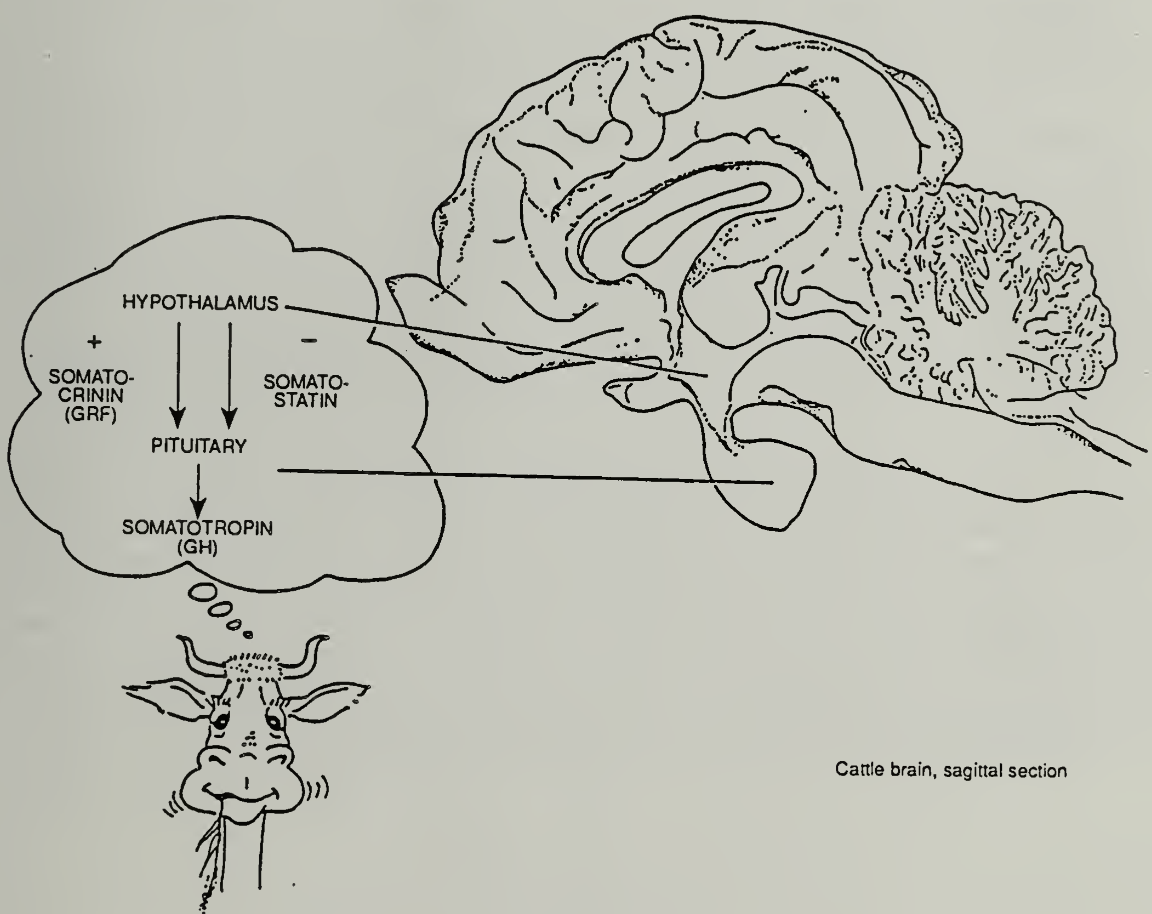

Fig. 1 Regulation of somatotropin secretion

2.1 SOMATOTROPIN IN THE BODY

ST plays a vital role in ensuring that the nutrients absorbed are efficiently used for muscular growth in the young animal or for milk production in the cow.

ST can be visualized as the manager of a processing plant (the dairy cow) who must ensure that inputs (feed) are directed to a processing unit (mammary gland) at the optimum rate to maximize production (milk) while maintaining or making judicious use of stocks (body reserves) during periods of supply deficit (beginning of lactation). The manager's drive, however, on which the plant's productivity is directly dependent, is influenced by the board of directors, particularly two powerful members, GRF and somatostatin.

Animals with greater potential for growth and milk production have, in fact, higher blood concentrations of ST during growth and lactation than animals with low genetic potential. Blood concentrations of ST are high when lactation begins and show a marked decline from the beginning to the end of lactation as milk production declines.

Results obtained in basic research thus suggested the idea that ST supplements might improve lactation performance in dairy cows.

2.2 METHODS OF INCREASING BLOOD CONCENTRATIONS OF SOMATOTROPIN

Blood concentrations of ST can be increased in five ways: (1) by genetic selection, which permits slight gradual increases in milk production, and thus ST, over a period of years; (2) by the administration of exogenous ST; (3) by stimulation of endogenous secretion of ST through the administration of exogenous GRF; (4) by vaccination against the inhibiting effect of somatostatin; or (5) by insertion in the genetic code of additional copies of the genes responsible for the synthesis of GRF or ST.

Bovine ST, a protein consisting of 190 or 191 amino acids in a sequence specific to the species, is currently produced by genetic engineering, like insulin, a hormone used in the therapeutic treatment of human diabetes. GRF, a much smaller molecule, can be produced in the laboratory by simple chemical synthesis. In recent years, the administration of exogenous ST or GRF has been tested intensively in lactating cows. Research is still continuing and these two molecules are not yet commercially available in Canada. Research on vaccination against somatostatin or manipulation of the genetic code is still in its infancy. The researchers at the Lennoxville Research Station have chosen to work on the GRF and somatostatin approaches.

2.3 SOMATOTROPIN AND LACTATION

Numerous studies over the past 50 years have demonstrated that bovine ST (bST) has a galactopoietic effect; that is, it can amplify an existing milk production. Azimov and Krouze (1937) were the first to stimulate milk production in dairy cows through the administration of a crude anterior pituitary extract in physiological saline. Dairy cows receiving subcutaneous (s.c.) injections of these extracts every two days for a period of three weeks produced 20% more milk (Folley and Young 1945). However, the galactopoietic effect of the pituitary extract, initially attributed to prolactin (PRL), was due instead to its ST content (Brumby and Hancock 1955; Cotes et al. 1949; Hutton 1957). In 1973, Machlin clearly demonstrated that a preparation of bST relatively free of PRL and thyrotropin increased milk production by 18% and consumption by 19% when injected every day for a period of 10 days. He also observed an increase in milk production of 5 kg per cow per day (35%) in cows receiving daily injections of a preparation of ST for eight weeks. However, the limited supply of bST available until very recently precluded the possibility of commercial applications of bST to improve the milk production of dairy cows.

The recent discovery of the recombinant DNA technique has made it possible to synthesize large quantities of recombinantly-derived bST. In a long-term study (84 to 272 days of lactation), Dale Bauman of Cornell University studied the effect of pituitary bST and recombinantly-derived bST on milk production and feed intake of high producing dairy cows (Bauman et al. 1985). Methionyl-bST increased milk production from 27.9 kg/day (controls) to 34.4, 38.0 and 39.4 kg/day in cows receiving daily s.c. injections of 13.5, 27.0 and 40.5 mg per injection, respectively. The increase in milk production varied from 23 to 41%, with no change in milk composition; unstimulated anticipated milk yield of these cows, based on pre-treatment estimates, was over 9600 kg. Milk production following injections of pituitary bST was lower than that produced by the administration of an equal dose of recombinantly-derived bST. The biological basis for this difference is not clear, according to Bauman et al. (1985), and remains unexplained at present. The cows receiving bST injections were able to maintain their physical condition over the entire treatment period, and increased their feed consumption to sustain the higher milk production. In addition, the cows treated with bST converted feed to milk more efficiently (8 to 24%), as indicated by the higher ratio of the number of kilograms of milk corrected for milk fat per megacalorie of feed consumed. On the whole, these results are extremely impressive. While there is certainly a plateau on milk production, there is no indication that it has yet been reached in dairy cows. In general, however, subsequent studies showed that treatment with bST increased milk yield by an average of 3 to 5 kg per day (review: Chilliard 1988).

3. SOMATOCRININ IN CATTLE

Since somatocrinin (GRF) increases endogenous secretions of somatotropin (ST), the biological effects of GRF should be similar to those of ST.

3.1 EFFECT ON SOMATOTROPIN SECRETION

It has been noted that the original molecule of GRF was discovered in man (Guillemin et al. 1982). The first step was to determine whether human (h) GRF(1-44)NH_2 was biologically active in cattle, since it was not until two years later that bovine GRF was sequenced (Guillemin et al. 1984). First of all, experiments were conducted to determine the optimal dose, frequency and route of injection and the nature of the peptide required to produce a satisfactory biological response in lactating cows (Petitclerc et al. 1985).

In an initial experiment, various doses of hGRF(1-44)NH_2 were compared for the ST secretion response. Nine Holstein cows between 30 and 60 days of lactation received an intravenous (i.v.) bolus of 0.5 mg of hGRF(1-44)NH_2 (0.2 nmol/kg body weight).

One week later, the same cows received an i.v. bolus of 2.0 mg of hGRF(1-44)NH_2 (0.8 nmol/kg). With the 0.2 nmol/kg dose, serum ST levels peaked at 10.8 ng/mL 120 minutes following the injection of hGRF(1-44)NH_2. Similarly, the injection of 0.8 nmol/kg raised serum ST levels to 13.4 ng/mL 180 minutes following the injection. The area under the ST curve, however, was different for the two doses (table 1).

Table 1 Areas under the ST curve (ng.min/mL) following injection of hGRF(1-44)NH_2 in dairy cows

Experiment No	Dose (μg/kg body weight)		
	Intravenous injection		Intramuscular injection
	1[1]	4	9
1	560	1250	
2			523
3	735 (10:00 a.m.) 468 (4:00 p.m.)		

[1] The 1 μg/kg dose of hGRF(1-44)NH_2 represents 0.2 nmol/kg body weight.

We can thus conclude that the higher dose of 0.8 nmol/kg, representing 4 μg of GRF(1-44)NH_2/kg body weight, permits greater secretion of ST than the 1 μg/kg dose after an i.v. injection.

The second experiment was designed to determine whether intramuscular (i.m.) injections of hGRF(1-44)NH_2 could increase ST secretion as well. Nine Holstein cows between 150 and 175 days of lactation received a saline solution or i.m. bolus of 5 mg of hGRF(1-44)NH_2 (1.8 nmol/kg body weight). Serum ST concentration peaked at 10.5 ng/mL 15 minutes following the injection. The area under the bST curve was 523 ng.min/mL (table 1). It should be noted that the quantity of ST secreted with the i.m. dose of 9 μg/kg is similar to that secreted with an i.v. injection of 1 μg/kg; in other words, an i.m. dose nine times larger than the i.v. dose produced the same ST response.

3.2 EFFECT ON MILK PRODUCTION

The third experiment was designed to determine whether the increase in ST obtained following the administration of GRF could increase milk production. Fifteen cows at an average of 186 days of lactation received 1 μg/kg i.v. injections of saline or GRF(1-44)NH_2 twice a day, at 10:00 a.m. and 4:00 p.m., for 10 consecutive days. ST concentrations rose at both injection times, from 6.4 to 12.9 and 9.6 ng/mL, respectively (table 1). The cows' milk production averaged 16.6 kg/day for the last five days of the injection period. The administration of GRF(1-44)NH_2 permitted a marginal increase in milk production of 4.8% after correction for persistency of lactation (Lapierre et al. 1985).

At the time, the difficulty involved in obtaining sufficient quantities of hGRF(1-44)NH_2 to perform the tests on dairy cows represented a major problem, since hGRF(1-44)NH_2 was very costly and very rare. A search therefore began for biologically active molecules which would be less expensive to produce. As a result, it was demonstrated that a fragment of GRF, hGRF(1-29)NH_2, had the same biological activity as hGRF(1-44)NH_2 on ST secretion in growing pigs and heifers (Petitclerc et al. 1987). The subsequent study was designed to compare the effect of hGRF(1-44)NH_2 and a fragment, hGRF(1-29)NH_2, on milk production (Pelletier et al. 1987). The GRF was administered for 10 days, six times a day, at a dose of 0.2 nmol/kg. The cows used were at an average of 158 days of lactation. The administration of hGRF(1-44)NH_2 or hGRF(1-29)NH_2 produced increases in milk production of 16.6 and 12.4%, respectively (table 2).

Table 2 Comparison of intravenous injections of hGRF(1-44)NH_2 and hGRF(1-29)NH_2 in doses of 0.2 nmol/kg six times a day for 10 consecutive days in dairy cows

Variable	Treatment[1]		
	Control	hGRF(1-44)NH_2	hGRF(1-29)NH_2
Milk production (kg/day)	23.4	27.3	26.3
Consumption of dry matter (kg/day)	19.8	19.8	19.9
Feed conversion (kg milk/kg dry matter consumed)	1.19	1.38	1.33

[1] The 0.2 nmol/kg dose represents 1 µg/kg for hGRF(1-44)NH_2 and 0.66 µg/kg for hGRF(1-29)NH_2.

The total yield of fat and protein increased with the hormone treatments as well. The fat and protein content of the milk also changed slightly in response to the treatment, since the cows received injections for only ten days and their metabolism did not have time to adjust. It is known, however, that prolonged injections of ST in dairy cows do not affect milk composition (review: Chilliard 1988).

In the dairy cow, hGRF(1-29)NH_2 and hGRF(1-44)NH_2 demonstrated similar biological activity. The intrinsic biological activity of hGRF(1-44)NH_2 on the release of ST thus lies in the sequence of the first 29 amino acids of the peptide. This experiment proved extremely valuable for subsequent studies since it justified the use of hGRF(1-29)NH_2, which was easier to synthesize and less expensive to produce than hGRF(1-44)NH_2. In addition, milk production increased in this experiment by 15 to 18%, compared to barely 5% in the previous experiment. It thus appears that twice-daily i.v. injections of hGRF(1-44)NH_2 at a dose of 0.2 nmol/kg body weight do not stimulate ST secretion enough to produce any substantial increase in milk production. Six i.v. injections a day, however, would be impractical.

The next step was to determine whether a single daily s.c. injection, even if it required more GRF than multiple i.v. injections, could produce a satisfactory biological response. The following experiment was therefore performed on 12 cows at an average of 209 days of lactation. Each of these cows received one daily s.c. injection of gelatin or 10 mg of hGRF(1-29)NH_2 (18 µg/kg) in gelatin for 10 days. The results were conclusive. The milk production of the treated cows increased by 3 kg/day (14.3%) for the last five days of treatment, with no change in milk fat or protein composition. Maximum

ST concentrations following injection of GRF averaged 34.1 ng/mL, compared to 2.9 ng/mL for the control cows (Lapierre et al. 1988a). This study demonstrated that a single daily injection of GRF could stimulate the endogenous secretion of ST enough to increase milk production.

Until this point, all the studies conducted using GRF at the Lennoxville Research Station had involved treatment periods of ten days. The next step was to determine whether long-term treatment was possible. Would continuous stimulation by the administration of exogenous GRF lead to desensitization of the pituitary gland? Previous studies with GRF did not suggest any such possibility, but the answer to this question held the key to future development of this technology in the area of animal production. The next experiment was therefore designed to determine the effect of GRF treatment for two months on 17 cows averaging 252 days of lactation at the beginning of the treatment. On completion of the treatment period, the cows were dried off. A preliminary test was conducted to determine whether the dose used in the preceding experiment produced optimal ST secretion. Doses of 5, 10 or 20 μg/kg produced statistically similar increases in ST concentrations (Lapierre et al. 1988b). Because the results produced by the 5 μg/mg dose were numerically slightly lower, however, the 10 μg/kg dose was selected.

For a period of 56 days, the cows received daily s.c. injections of saline or 10 μg/kg of hGRF(1-29)NH_2. After two months of treatment, the maximum concentration of ST achieved following the injection of GRF was higher than on the first injection, at 46.8 compared to 25.2 ng/mL respectively, while the maximum concentrations achieved in the control cows averaged 3.6 ng/mL. In addition, at the end of the treatment period, all the cows received an injection of GRF (1 μg/kg i.v.) to determine clearly whether two months of treatment had influenced the cows' ability to respond to GRF injections. The cows which had received no previous injections of GRF produced maximum concentrations slightly lower than those which had received daily injections of GRF for two months, at 21.8 compared to 32.2 ng/mL; the areas under the ST response curve, however, showed no difference (Lapierre et al. 1988b). In terms of milk yield following this treatment, treatment with GRF led to a less rapid decline in production at the end of lactation, resulting in a mean increase of 1.9 kg/day over the treatment period, with the control cows producing 13.9 kg/day over their 60 days of lactation. On the subsequent lactation, calf weight and milk production were recorded. GRF treatment at the end of the previous lactation did not affect these variables (Lapierre et al. 1988c). This experiment clearly demonstrated that long-term treatment with GRF was possible with no risk of pituitary desensitization, since the cows responded just as well after two months of injections as on the initial administration, if not better.

As mentioned previously, endogenous secretion of ST following the injection of GRF reaches a plateau at high doses of GRF. This means that, above a certain dose of GRF, 10 μg/kg s.c. in the dairy cow, the quantity of ST secreted will not increase even if the quantity of GRF administered is increased. It had been demonstrated that hGRF(1-44)NH_2 was degraded in one minute to hGRF(3-44)NH_2, a peptide with only 1/100 the activity of the parent molecule (Frohman et al.1986). Substitution of amino acids could produce an analogue resistant to the peptide degradation of amino acids 1-3. As a result,

some study was given to the substitution of amino acids to improve the helical amphiphilic structure of GRF. An analogue of hGRF(1-29)NH_2 containing three substitutions was then synthesized (Felix et al. 1986). First of all, tyrosine and alanine, in positions 1 and 2 respectively, were replaced by a desamino-tyrosine and a D-alanine to reduce the peptide's sensitivity to peptidases. Secondly, the glycine in position 15 was replaced by an alanine, to permit better insertion in the hydrophobic region of the molecule. The use of a GRF analogue permitted more effective stimulation of endogenous ST secretion. It remained to be determined how this analogue would behave in the dairy cow and whether increases in ST secretion beyond those previously obtained would be reflected by corresponding increases in milk production.

The next step was to determine the dose of the analogue to be used. We compared the ST response at different doses of hGRF(1-29)NH_2 (3.3 and 10 μg/kg) and at different analogue doses (0.37, 1.11 and 3.33 μg/kg). The responses to the different GRFs are shown in figure 2. By deduction, an analogue dose of 0.6 μg/kg should produce endogenous secretion of ST similar to the administration of 10 μg/kg of hGRF(1-29)NH_2. This dose was therefore selected for the next experiment on milk production, together with a dose three times as large (1.8 μg/kg), and compared with our usual dose, 10 μg/kg of hGRF(1-29)NH_2. For ten days, twenty-four cows received daily s.c. injections of saline, hGRF(1-29)NH_2 (10 μg/kg) or an analogue containing three substitutions (0.6 or 1.8 μg/kg; n = 6 per treatment). The ST concentrations in response to the different GRF treatments were similar, with mean maximum concentrations of 40.1 ng/mL, compared to maximum concentrations of 3.3 ng/mL in the control cows (figure 3). It should be noted, however, that even after eight hours of blood sampling, ST concentrations in the cows receiving the highest dose of the analogue had not returned to the basal level. Milk yields increased by 1.8, 2.2 and 3.1 kg/day in response to hGRF(1-29)NH_2 and GRF analogue in doses of 0.6 μg/kg and 1.8 μg/kg respectively, with the control cows producing 18.8 kg/day (figure 3). The increases obtained in response to treatment with hGRF and with the lower dose of the analogue were similar, while the higher dose of the analogue resulted in a further increase in milk production (Lapierre et al. 1990a). This experiment indicates that the analogue with the three substitutions generates the same increase in dairy production as hGRF(1-29)NH_2, but with 1/16 the dose.

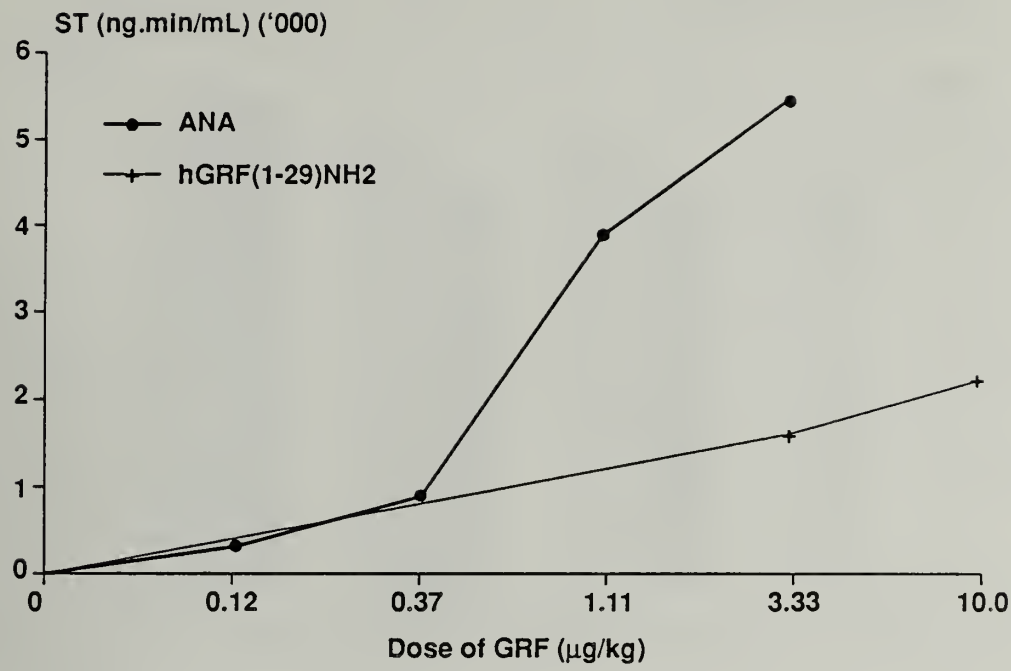

Fig. 2 Effect of different doses of somatocrinin (hGRF(1-29)NH_2) or an analogue (ANA) on the secretion of somatotropin (ST) in dairy cows

In a final experiment, cows received long-term treatment for a period of 182 days, from 120 days of lactation to drying off. They received daily injections of hGRF(1-29)NH_2 (10 µg/kg, s.c.). GRF increased milk production by 9.5% and feed efficiency by 6.1% with no change in milk composition (Lacasse et al. 1991a). The ST response to GRF injections persisted and even increased over the injection period (Lacasse et al. 1991b).

In summary, following a demonstration of hGRF(1-44)NH_2 activity in dairy cattle, it was established that the fragment containing the first 29 amino acids of the original molecule was equipotent to the parent molecule on ST secretion and milk production. In addition, the effect of GRF on ST secretion and, indirectly, on milk production, persisted over long-term treatment periods varying from 60 to 182 days. Finally, synthesis of a GRF analogue containing substitutions of amino acids to improve the half-life and structure of the GRF permitted the use of much smaller doses of peptides and even larger increases in milk production. GRF has thus been shown to be a tool with the potential to increase milk yield and feed efficiency in dairy cows.

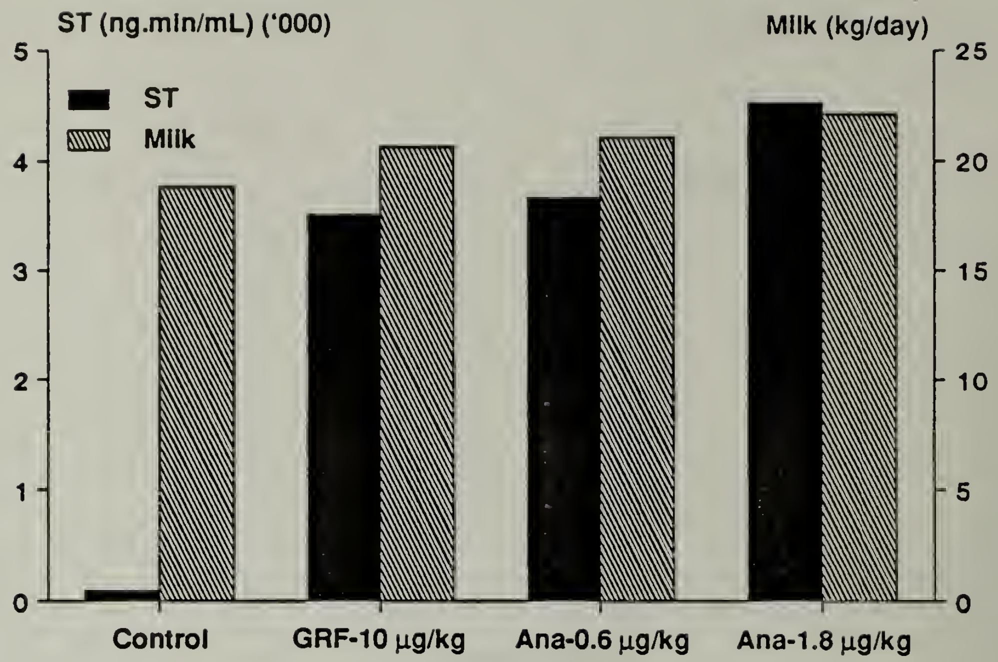

Fig. 3 Effect of somatocrinin (GRF) or an analogue (ANA) on the secretion of somatotropin (ST) and milk production in dairy cows

3.3 EFFECT ON THE DEVELOPMENT OF THE MAMMARY GLAND

The weight or volume of parenchymal epithelial tissue in the mammary gland can be increased by 30 to 45% by injecting prepubertal heifers with bovine ST (Sejrsen et al. 1986; Sandles and Peel 1987) or with GRF to increase ST concentrations (Ringuet et al. 1989). These treatments are accompanied by a significant reduction in the quantity of adipose tissue in the mammary gland. Milk production in heifers receiving this treatment does not increase, according to Sandles and Peel (1987); in this study, however, the heifers growth over the treatment period was very low (less than 400 g/day). Sejrsen, on the other hand, reports an increase in milk production of approximately 8% in heifers with feeding levels permitting growth rates of 700 or over 1000 g per day (personal communication).

3.4 EFFECT ON GROWTH AND METABOLISM

ST brings about much more spectacular biological responses in lactating cows than in growing cattle. In general, ST treatment increases nitrogen retention (Moseley et al. 1982, 1987; Crooker et al. 1990) by diverting energy towards protein and away from fat (Eisemann et al. 1986). Except in one experiment (Moseley et al. 1982), dietary digestibility was not affected.

The effect of GRF on growing cattle was studied first in grain-fed calves. A grain-fed calf is a young bovine which receives milk-replacer for approximately six weeks and is then weaned; it receives cereal-based concentrates and protein supplements ad libitum from the age of two weeks until slaughter. After weaning, 30 male dairy calves with a mean live weight of 70 kg received two s.c. injections a day of saline or hGRF(1-29)NH_2, at a dose of 5 μg/kg per injection, until slaughter, at a live weight of 220 kg (123 days of treatment). The ST response to GRF injections declined with age. This decline was not due to the treatment with GRF since, after 87 days of treatment, each animal received an injection of GRF and the control animals showed exactly the same response as those which had been previously treated (Lapierre et al. 1990b). In contrast to the observations noted with ST, dietary digestibility increased with GRF treatment. Nitrogen retention also increased slightly. This increase in nitrogen retention was not reflected, however, in a change in body composition or an increase in the animals' growth. The feed efficiency of animals treated with GRF and those which were untreated was identical (Lapierre et al. 1991). GRF treatment of young growing cattle, while it increased the quantity of nitrogen retained by the animal, did not affect their growth performance or body composition.

In a second experiment on growing cattle, we studied the effect of GRF on energy and splanchnic metabolism (gastrointestinal system and liver). Beef steers, weighing an average of 339 kg, received two daily s.c. injections of saline or hGRF(1-29)NH_2 (10 μg/kg per injection) and one of two feeding treatments (a moderate quantity just above maintenance level and a level 1.8 times as high); treatment continued for 21 days. Catheters were inserted in the portal and hepatic veins and caudal artery to permit simultaneous blood sampling at these three sites, and blood flow was measured by an infusion of para-aminohippuric acid.

Treatment with GRF increased nitrogen retention by 108% in the animals receiving the moderate feed level and by 83% in those receiving the high feed level. As observed earlier, GRF increased dietary digestibility. The energy required for maintenance was not affected by GRF. The efficiency with which the animal uses the metabolizable energy for retention in its tissues did not change. The amount of energy retained by the animals was not affected by GRF but the partitioning of this energy changed significantly, with energy being diverted towards protein and away from fat (figure 4; Lapierre et al. 1992). Catabolism of amino acids and production of urea by the liver, like the urinary excretion of nitrogen, were reduced by GRF treatment (Reynolds et al. 1992). This experiment clearly indicates the coordination between the different organs in responding to the metabolic changes brought about by treatment with GRF.

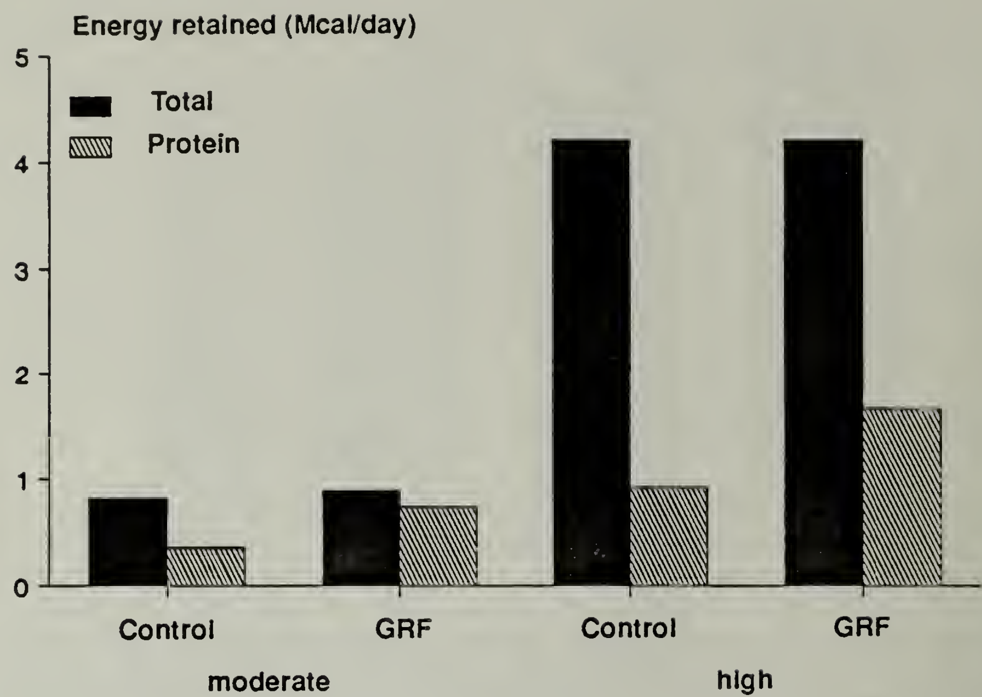

Fig. 4 Effect of somatocrinin on energy retention in the tissues and on energy partitioning in growing steers fed two different intake levels, moderate or higher

It has been noted that the secretion of ST is controlled by two hypothalamic factors: GRF, which stimulates the secretion of ST, and somatostatin, which inhibits it. The studies presented above involved the use of GRF to increase endogenous secretions of ST and thus animal performance. The degree of improvement in performance appears to be related to the level of the increase in ST as a result of GRF injections.

However, the injection of GRF, which acts as an accelerator, does not eliminate somatostatin, which acts as a brake. The following hypothesis was therefore proposed. If the brake (somatostatin) could be released and the accelerator (GRF) activated, ST secretion might increase even more in response to lower doses of GRF. In addition, inhibition of the brake alone might be enough to increase ST secretion. Studies by other investigators indicate that this may be a promising approach. The basic principle of immunoneutralization of somatostatin is similar to that of vaccination against disease, except that the antibodies which the animal produces are directed against endogenous

somatostatin rather than a virus or bacteria.

An experiment was conducted on grain-fed calves to determine the effect of GRF infusion and active immunization against somatostatin on animal growth, carcass quality, weight of the digestive organs and hormone secretion. Thirty-two male calves were divided into two uniform groups, with half being immunized against human alpha-globulin and the other half against somatostatin conjugated with human alpha-globulin, one week after weaning (79 kg). Since somatostatin is a small molecule, it must be combined with an antigen to encourage the formation of antibodies. Boosters were administered on days 14, 28, 56 and 73 of the experimental period. The calves immunized against somatostatin developed significantly more antibodies specifically against somatostatin than the control calves. Between days 79 and 111, half of the calves from each of the immunization groups were infused with hGRF(1-29)NH_2 at a dose of 3.33 ng.min/kg of body weight. The animals were slaughtered at days 119 and 128 to obtain data on carcass quality and the weight of various organs.

The desired results were not achieved: immunization against somatostatin reduced the concentrations of ST (8.3 compared to 16.1 ng/mL) and insulin-like growth factor 1 (126.0 compared to 150.9 ng/mL). Two possible explanations were proposed. First, the clearance speed of these hormones may have increased as a result of immunization against somatostatin, thus reducing blood concentrations, as Kenison (1987) has suggested. Another possibility is that immunization against somatostatin led to the development of antiidiotypes, substances acting as antibodies against the antibodies which inhibit somatostatin. Their biological effect could even be similar to that of somatostatin (image-like antiidiotypes), which inhibits the secretion of ST. As in earlier studies, GRF treatment caused an increase in blood concentrations of ST and insulin-like growth factor 1 (Roy et al. 1989).

The effect of the hormone treatments on the calves' performance is related to the concentrations of ST and insulin-like growth factor 1. In fact, GRF increased the daily gain in body weight, while immunization against somatostatin reduced it. Carcass composition and the weight of various organs (reticulo-rumen, omasum, abomasum, duodenum, small and large intestines, lungs, kidneys, spleen, thymus and testicles) were not affected by the treatments, with the exception of liver and pancreas weight, which decreased following immunization against somatostatin (Roy et al. 1990).

This experiment demonstrated that GRF specifically increases the activity of pancreatic amylase (Roy et al. 1991). This action may have a beneficial effect on the digestion of starch in ruminants.

GRF thus influences the metabolism of growing cattle by increasing the quantity of energy retained in the form of protein without affecting total energy retention. In addition, GRF increased dietary digestibility in two experiments and did not affect it when infused. This effect, which differs from that of ST, requires further study. Immunization against somatostatin did not prove, at least initially as promising.

4. CONCLUSION

We at the Lennoxville Research Station have chosen to concentrate on the use of somatocrinin to increase somatotropin concentrations in order to improve production efficiency in growing or lactating cattle. Somatocrinin was first characterized barely ten years ago. In this technical document, we have described the progress which we have made in this short time, moving from the newly discovered molecule to a final demonstration of its potential.

The mechanisms involved in the increased milk production in dairy cows and nitrogen retention in growing cattle following treatment with somatocrinin have not yet been fully explained. However, it is becoming increasingly clear that the entire organism is implicated in this metabolic change: the various organs or tissues act together to divert nutrients towards sites of increased demand and to reduce the use of these nutrients by other sites. The somatotropic axis appears to play a leading role in the orchestration of the metabolism for high producing animals. The insulin-like growth factor 1, which show higher blood concentrations following GRF treatment (Abribat et al. 1990; Lapierre et al. 1990a, 1991, 1992b), but which have now been shown to display paracrine activity as well, complete the series of events associated with the somatotropic axis. However, their exact mode of action and the effect of their binding proteins require further clarification. It also remains to be determined whether the effect of somatocrinin in increasing dietary digestibility is indeed real and, if so, why it acts differently from somatotropin in this context. Research is still required for a better understanding of the mechanisms involved in the metabolic coordination required of animals to increase their bioenergetic efficiency.

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